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<b>(54) Title:</b> HUMAN CALCIUM CHANNELS ALFA1 SUBUNITS AND RELATED PROBES, CELL LINES AND METHODS			
<b>(57) Abstract</b>  Partial sequences for a novel mammalian (human and rat sequences identified) calcium channel subunit which we have labeled as the $\alpha_{II}$ subunit, and an additional novel human calcium channel which we have labeled as the $\alpha_{IH}$ subunit are provided. Knowledge of the sequence of these two calcium channels permits the localization and recovery of the complete sequence from human cells, and the development of cell lines which express the novel calcium channels of the invention. These cells may be used for identifying compounds capable of acting as agonists or antagonists to the calcium channels.			

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- 1 -

# HUMAN CALCIUM CHANNELS ALPHA1 SUBUNITS AND RELATED PROBES, CELL LINES AND METHODS

## DESCRIPTION

### TECHNICAL FIELD

The present invention relates to novel human calcium channel compositions, and to the expression of these compositions in cell lines for use in evaluating calcium channel function.

### BACKGROUND OF THE INVENTION

The rapid entry of calcium into cells is mediated by a class of proteins called voltage-gated calcium channels. Calcium channels are a heterogeneous class of molecules that respond to depolarization by opening a calcium-selective pore through the plasma membrane. The entry of calcium into cells mediates a wide variety of cellular and physiological responses including excitation-contraction coupling, hormone secretion and gene expression. In neurons, calcium entry directly affects membrane potential and contributes to electrical properties such as excitability, repetitive firing patterns and pacemaker activity. Miller, R.J. (1987) Multiple calcium channels and neuronal function. Science 235:46-52. Calcium entry further affects neuronal functions by directly regulating calcium-dependent ion channels and modulating the activity of calcium-dependent enzymes such as protein kinase C and calmodulin-dependent protein kinase II. An increase in calcium concentration at the presynaptic nerve terminal triggers the release of neurotransmitter. Calcium entry also plays a role in neurite outgrowth and growth cone migration in developing neurons and has been implicated in long-term changes in neuronal activity. In addition to the variety of normal physiological functions mediated by calcium channels, they are also implicated in a number of human disorders. Recently, mutations identified in human and mouse calcium channel genes have been found to account for several disorders including, familial hemiplegic migraine, episodic ataxia type 2, cerebellar ataxia, absence epilepsy and seizures. Fletcher, et al. (1996) Absence epilepsy in tottering mutant mice is associated with calcium channel defects. Cell

- 2 -

87:607-617; Burgess, et al. (1997) Mutation of the Ca<sup>2+</sup> channel  $\beta$  subunit gene *Cchb4* is associated with ataxia and seizures in the lethargic (lh) mouse. *Cell* 88:385-392; Ophoff, et al. (1996) Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca<sup>2+</sup> channel gene *CACNL1A4*. *cell* 87:543-552; Zhuchenko, O. et al. (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with the small polyglutamine expansions in the  $\alpha 1A$ -voltage-dependent calcium channel. *Nature Genetics* 15:62-69.

The clinical treatment of some disorders has been aided by the development of therapeutic calcium channel antagonists. Janis, et al. (1991) In *Calcium Channels: Their Properties, Functions, Regulation and Clinical Relevance*. CRC Press, London.

Native calcium channels have been classified by their electrophysiological and pharmacological properties as T, L, N, P and Q types (for reviews see McCleskey, et al. (1991) Functional properties of voltage-dependent calcium channels. *Curr. Topics Membr.* 39: 295-326, and Dunlap, et al. (1995) Exocytotic Ca<sup>2+</sup> channels in mammalian central neurons. *Trends Neurosci.* 18:89-98.). T-type (or low voltage-activated) channels describe a broad class of molecules that transiently activate at negative potentials and are highly sensitive to changes in resting potential. The L, N, P and Q-type channels activate at more positive potentials and display diverse kinetics and voltage-dependent properties. There is some overlap in biophysical properties of the high voltage-activated channels, consequently pharmacological profiles are useful to further distinguish them. L-type channels are sensitive to dihydropyridine (DHP) agonists and antagonists, N-type channels are blocked by the *Conus geographus* peptide toxin,  $\omega$ -conotoxin GVIA, and P-type channels are blocked by the peptide  $\omega$ -agatoxin IVA from the venom of the funnel web spider, *Agelenopsis aperta*. A fourth type of high voltage-activated Ca channel (Q-type) has been described, although whether the Q- and P-type channels are distinct molecular entities is controversial (Sather et al. (1993) Distinctive biophysical and pharmacological properties of class A (B1) calcium channel  $\alpha 1$  subunits. *Neuron* 11: 291-303; Stea, et al. (1994) Localization and functional properties of a rat brain  $\alpha 1A$  calcium channel reflect similarities to neuronal Q- and P-type channels. *Proc Natl Acad Sci (USA)* 91: 10576-10580.). Several types of calcium conductances do not fall

- 3 -

neatly into any of the above categories and there is variability of properties even within a category suggesting that additional calcium channels subtypes remain to be classified.

Biochemical analyses show that neuronal calcium channels are heterooligomeric complexes consisting of three distinct subunits ( $\alpha_1$ ,  $\alpha_2\delta$  and  $\beta$ ) (reviewed by De Waard, et al. (1997) In Ion Channels. Volume 4. edited by Narahashi, T. Plenum Press, New York). The  $\alpha_1$  subunit is the major pore-forming subunit and contains the voltage sensor and binding sites for calcium channel antagonists. The mainly extracellular  $\alpha_2$  is disulphide-linked to the transmembrane  $\delta$  subunit and both are derived from the same gene and are proteolytically cleaved *in vivo*. The  $\beta$  subunit is a non-glycosylated, hydrophilic protein with a high affinity of binding to a cytoplasmic region of the  $\alpha_1$  subunit. A fourth subunit,  $\gamma$ , is unique to L-type Ca channels expressed in skeletal muscle T-tubules. The isolation and characterization of  $\gamma$ -subunit-encoding cDNAs is described in US Patent No. 5,386,025 which is incorporated herein by reference.

Molecular cloning has revealed the cDNA and corresponding amino acid sequences of six different types of  $\alpha_1$  subunits ( $\alpha_{1A}$ ,  $\alpha_{1B}$ ,  $\alpha_{1C}$ ,  $\alpha_{1D}$ ,  $\alpha_{1E}$  and  $\alpha_{1S}$ ) and four types of  $\beta$  subunits ( $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  and  $\beta_4$ ) (reviewed in Stea, A., Soong, T.W. and Snutch, T.P. (1994) Voltage-gated calcium channels. PCT Patent Publication WO 95/04144, which is incorporated herein by reference, discloses the sequence and expression of  $\alpha_{1E}$  calcium channel subunits. In Handbook of Receptors and Channels. Edited by R.A. North, CRC Press.).

The different classes of  $\alpha_1$  and  $\beta$  subunits have been identified in different animals including, rat, rabbit and human and share a significant degree of amino acid conservation across species (for examples see: Castellano, et al. (1993) Cloning and expression of a third calcium channel  $\beta$  subunit. J. Biol. Chem. 268: 3450-3455; Castellano, et al. (1993) Cloning and expression of a neuronal calcium channel  $\beta$  subunit. J. Biol. Chem. 268: 12359-12366; Dubel, et al. (1992). Molecular cloning of the  $\alpha_1$  subunit of an  $\omega$ -conotoxin-sensitive calcium channel. Proc. Natl. Acad. Sci. (USA) 89: 5058-5062; Fujita, et al.. (1993) Primary structure and functional expression of the  $\omega$ -conotoxin-sensitive N-type calcium channel from rabbit brain. Neuron 10: 585-598; Mikami, et al.. (1989). Primary structure and functional

- 4 -

expression of the cardiac dihydropyridine-sensitive calcium channel. *Nature* 340: 230-233; Mori, et al. (1991) Primary structure and functional expression from complementary DNA of a brain calcium channel. *Nature* 350: 398-402; Perez-Reyes, et al. (1992). Cloning and expression of a cardiac/brain  $\beta$  subunit of the L-type calcium channel. *J. Biol. Chem.* 267: 1792-1797; Pragnell, et al. (1991). Cloning and tissue-specific expression of the brain calcium channel  $\beta$ -subunit. *FEBS Lett.* 291: 253-258; Snutch, et al. (1991) Distinct calcium channels are generated by alternative splicing and are differentially expressed in the mammalian CNS. *Neuron* 7: 45-57; Soong, et al. (1993) Structure and functional expression of a member of the low voltage-activated calcium channel family. *Science* 260: 1133-1136; Tomlinson, et al. (1993) Functional properties of a neuronal class C L-type channel. *Neuropharmacology* 32: 1117-1126; Williams, et al. (1992) Structure and functional expression of  $\alpha 1$ ,  $\alpha 2$ , and  $\beta$  subunits of a novel human neuronal calcium channel subtype. *Neuron* 8: 71-84; Williams, et al. (1992) Structure and functional expression of an  $\omega$ -conotoxin-sensitive human N-type calcium channel. *Science* 257: 389-395.

In some expression systems the  $\alpha_1$  subunits alone can form functional calcium channels although their electrophysiological and pharmacological properties can be differentially modulated by coexpression with any of the four  $\beta$  subunits. Until recently, the reported modulatory affects of  $\beta$  subunit coexpression were to mainly alter kinetic and voltage-dependent properties. More recently it has been shown that  $\beta$  subunits also play crucial roles in modulating channel activity by protein kinase A, protein kinase C and direct G-protein interaction. (Bourinet, et al. (1994) Voltage-dependent facilitation of a neuronal  $\alpha 1C$  L-type calcium channel. *EMBO J.* 13: 5032-5039; Stea, et al. (1995) Determinants of PKC-dependent modulation of a family of neuronal calcium channels. *Neuron* 15:929-940; Bourinet, et al. (1996) Determinants of the G-protein-dependent opioid modulation of neuronal calcium channels. *Proc. Natl. Acad. Sci. (USA)* 93: 1486-1491.)

The electrophysiological and pharmacological properties of the calcium channels cloned to date can be summarized as shown in Table 1. While the cloned  $\alpha_1$  subunits identified to date correspond to several of the calcium channels found in cells, they do not account for all types of calcium conductances described in native cells. For example,

- 5 -

they do not account for the various properties described for the heterogenous family described as T-type calcium channels. Furthermore, they do not account for novel calcium channels described in cerebellar granule cells or other types of cells. ( Forti, et al (1993) Functional diversity of L-type calcium channels in rat cerebellar neurons. Neuron 10: 437-450; Tottene, et al. (1996). Functional diversity of P-type and R-type calcium channels in rat cerebellar neurons. J. Neurosci. 16: 6353-6363).

Because of the importance of calcium channels in cellular metabolism and human disease, it would be desirable to identify the remaining classes of  $\alpha_1$  subunits, and to develop expression systems for these subunits which would permit the study and characterization of these calcium channels, including the study of pharmacological modulators of calcium channel function. Thus, it is an object of the present invention to provide heretofor undisclosed calcium channels having novel  $\alpha_1$  subunits, including cell lines expressing these new calcium channels. It is a further object of the present invention to provide a method for testing these novel calcium channels using such cell lines.

## SUMMARY OF THE INVENTION

The present invention provides partial sequences for a novel mammalian (human and rat sequences identified) calcium channel subunit which we have labeled as the  $\alpha_{11}$  subunit, and an additional novel human calcium channel which we have labeled as the  $\alpha_{1H}$  subunit. This knowledge of the sequence of these two calcium channels permits the localization and recovery of the complete sequence from human cells, and the development of cell lines which express the novel calcium channels of the invention. These cells may be used for identifying compounds capable of acting as agonists or antagonists to the calcium channels.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows aligned amino acid sequences for the *C. elegans* C54D2.5  $\alpha_1$  calcium channel subunit and initially identified portions of the calcium channel subunits of the invention.

- 6 -

TABLE I						
	$\omega$ -conotoxin GVIA	1,4- dihydropyridines	cadmium	$\omega$ -agatoxin IVA	$\omega$ -conotoxin MVIIC	native Ca <sup>2+</sup> channel type
$\alpha_{1A}$	-	-	✓	✓	✓	P/Q-type
$\alpha_{1B}$	✓	-	✓	-	✓	N-type
$\alpha_{1C}$	-	✓	✓	-	-	L-type
$\alpha_{1D}$	-	✓	✓	-	-	L-type
$\alpha_{1E}$	-	-	✓	-	-	novel
$\alpha_{1S}$	-	✓	✓	-	-	L-type

## DESCRIPTION OF THE INVENTION

The present invention includes the following aspects for which protection is sought:

(a) novel human calcium channel subunits and DNA fragments encoding such subunits. It will be appreciated that polymorphic variations may be made or may exist in the DNA of some individuals leading to minor deviations in the DNA or amino acids sequences from those shown which do not lead to any substantial alteration in the function of the calcium channel. Such variations, including variations which lead to substitutions of amino acids having similar properties are considered to be within the scope of the present invention.

(b) polynucleotide sequences useful as probes in screening human cDNA libraries for genes encoding these novel calcium channel subunits. These probes can also be used in histological assay to determine the tissue distribution of the novel calcium channel subunits.

(c) eukaryotic cell lines expressing the novel calcium channel subunits. These cell lines can be used to evaluate compounds as pharmacological modifiers of the function of the novel calcium channel subunits.



- 7 -

(d) a method for evaluating compounds as pharmacological modifiers of the function of the novel calcium channel subunits using the cell lines expressing those subunits alone or in combination with other calcium channel subunits.

Further, since defects in the novel calcium channel subunits may be associated with a human genetic disease including, but not limited to; epilepsy, migraine, ataxia, schizophrenia, hypertension, arrhythmia, angina, depression, small lung carcinoma, Lambert-Eaton syndrome, characterization of such associations and ultimately diagnosis of associated diseases can be carried out with probes which bind to the wild-type or defective forms of the novel calcium channels.

In accordance with the present invention, we have identified human DNA sequences which code for novel calcium channel  $\alpha_1$  subunits. These subunits are believed to represent two new types of  $\alpha_1$  subunits of human voltage-dependent calcium channels which have been designated as type  $\alpha_{11}$  and type  $\alpha_{1H}$ .

The novel  $\alpha_1$  subunits of the invention were identified by screening the *C. elegans* genomic DNA sequence data base for sequences homologous to previously identified mammalian calcium channel  $\alpha_1$  subunits. Specifically, the following twelve mammalian  $\alpha_1$  subunit sequences were used to screen the *C. elegans* genomic data bank:

rat brain $\alpha_{1A}$ : GTCAAACTC AGGCCTTCTA CTGG	SEQ ID. No. 1
rat brain $\alpha_{1A}$ : AACGTGTTCT TGGCTATCGC GGTG	SEQ ID. No. 2
rat brain $\alpha_{1B}$ : GTGAAAGCAC AGAGCTTCTA CTGG	SEQ ID. No. 3
rat brain $\alpha_{1B}$ : AACGTTTTCT TGGCCATTGC TGTG	SEQ ID. No. 4
rat brain $\alpha_{1C}$ : GTTAAATCCA ACGTCTTCTA CTGG	SEQ ID. No. 5
rat brain $\alpha_{1C}$ : AATGTGTTCT TGGCCATTGC GGTG	SEQ ID. No. 6
rat brain $\alpha_{1D}$ : GTGAAGTCTG TCACGTTTTA CTGG	SEQ ID. No. 7
rat brain $\alpha_{1D}$ : AAGCTCTTCT TGGCCATTGC TGTA	SEQ ID. No. 8
rat brain $\alpha_{1E}$ : GTCAAGTCGC AAGTGTTCTA CTGG	SEQ ID. No. 9
rat brain $\alpha_{1E}$ : AATGTATTCT TGGCTATCGC TGTG	SEQ ID. No. 10
rat brain consensus #1 : ATCTAYGCYR TSATYGGSAT G	SEQ ID. No. 11

- 8 -

rat brain consensus #2 : ATGGACAAAT TYGASTAYTC

SEQ ID. No. 12

This search identified four distinct *C. elegans* cosmids that contain open reading frames (coding regions) that exhibit homology to mammalian calcium channel  $\alpha_1$  subunits:

cosmid and reading frame T02C5.5

cosmid and reading frame C48A7.1

cosmid and reading frame C54D2.5

cosmid and reading frame C27F2.3

Examination of the four *C. elegans* cosmid sequences by phylogeny analysis shows that two of these, T02C5.5 and C48A7.1, correspond closely with previously identified mammalian  $\alpha_1$  subunits. T02C5.5 appears to be an ancestral member related to the mammalian  $\alpha_{1A}$ ,  $\alpha_{1B}$  and  $\alpha_{1E}$  subunits. C48A7.1 appears to be an ancestral member related to the mammalian L-type channels encoded by  $\alpha_{1C}$ ,  $\alpha_{1D}$  and  $\alpha_{1S}$ . In contrast, the *C. elegans* cosmids C54D2.5 and C27F2.3 identify novel types of calcium channel  $\alpha_1$  subunits distinct from the other mammalian subtypes.

Mammalian counterparts of the *C. elegans* calcium channel  $\alpha_1$  subunit encoded by C54D2.5 were identified by screening of the GenBank expressed sequence tag (EST) data bank. This analysis identified a total of 13 mammalian sequences that exhibit some degree of DNA sequence and amino acid identity to C54D2.5, of which 8 are human sequences. (Table 2) Three of these sequences appear unlikely to encode novel calcium channel subunits because they either exhibit a significant degree of homology to previously identified mammalian  $\alpha_1$  subunits (clones H06096 and H14053) or exhibit homology in a region not considered to be diagnostic of calcium channel  $\alpha_1$  subunits specifically as opposed to other types of ion channel molecules in general (clone D20469). The five remaining sequences (H55225, H55617, H55223, H55544, and F07776), however, are believed to encode two previously unidentified calcium channel  $\alpha_1$  subunits because the degree of amino acid identity closely matches that of known calcium channel subunits in conserved regions but is sufficiently different to indicate that they do not encode previously identified mammalian

- 9 -

calcium channel  $\alpha_1$  subunits.  $\alpha_{1A}$ ,  $\alpha_{1B}$ ,  $\alpha_{1C}$ ,  $\alpha_{1D}$ ,  $\alpha_{1E}$ , or  $\alpha_{1S}$ . The expected amino acid sequence closely matches but is not identical to amino acid sequences in these known calcium channel subunits. The aligned amino acids sequences are shown in Fig 1.

Table 2

Query = C54D2.5 CE02562 CALCIUM CHANNEL ALPHA-1 SUBUNIT LG:6

Database: Non-redundant Database of GenBank EST Division

824,500 sequences; 302,742,428 total letter

Sequences producing High-scoring Segment Pairs:      Frame Score    P(N)

gb AA183990 AA183990	ms53e02.r1 Life Tech mouse embry...	+1	108	1.8e-24
gb H55225 H55225	CHR220164 Homo sapiens genomic c...	+1	136	2.5e-10
dbj D68412 CELK131B1F	C.elegans cDNA clone yk131b1 : 5...	+3	117	1.7e-06
gb R75128 R75128	MDB1075 Mouse brain, Stratagene ...	+3	113	7.2e-06
gb H55617 H55617	CHR220556 Homo sapiens genomic c...	+2	102	2.8e-05
emb F07776 HSC2HD061	H. sapiens partial cDNA sequence...	+3	100	0.00057
gb W76774 W76774	me84e08.r1 Soares mouse embryo N...	+2	98	0.0012
gb H06096 H06096	yl77e01.r1 Homo sapiens cDNA clo...	+3	98	0.0015
gb H14053 H14053	ym65d10.r1 Homo sapiens cDNA clo...	+2	91	0.0036
gb H55223 H55223	CHR220162 Homo sapiens genomic c...	+2	87	0.0039
dbj D35703 CELK024D9F	C.elegans cDNA clone yk24d9 : 5'...	+3	74	0.046
dbj D20469 HUMGS01443	Human HL60 3'directed MboI cDNA,...	-2	66	0.91
gb H55544 H55544	CHR220483 Homo sapiens genomic c...	+1	65	0.98

Four of the five sequences (H55225, H55617, H55223, and H55544) are found on human chromosome 22, and are now believed to all be part of the same gene encoding the

- 10 -

novel human calcium channel subunit  $\alpha_{11}$ . The fifth sequence, F07776 is apparently distinct and associated with a further novel human calcium channel subunit designated  $\alpha_{1H}$ .

The sequences of the five selected sequences and the references from which they are taken are given as follows:

H55225 SOURCE human clone=C22\_207 primer=T3 library=Chromosome 22  
exon

Trofatter, et al., *Genome Res.* 5 (3): 214-224 (1995)

SEQ ID No. 13

1 GTGATCACTC TGGAAGGCTG GGTGGAGATC ATGTACTACG TGATGGATGC  
TCACTCCTTC

61 TACAACTTCA TCTACTTCAT CCTGCTTATC ATACCCCTCT TGCCTTGCAC CCCATATGGT  
121 CTTCCCAGAG TGAGCTCATC CACCTCGTCA TGCCTGACTC GACGTTCA

H55617 SOURCE human clone=C22\_757 primer=T3 library=Chromosome 22  
exon

Trofatter, et al., *Genome Res.* 5 (3): 214-224 (1995)

SEQ ID No. 14

1 GATGGTCGAG TACTCCCTGG ACCTTCAGAA CATCAACCTG TCAGCCATCC  
GCACCGTGCG

61 CGTCCTGAGG CCCCTCAAAG CCATCAACCG CGTGCCCA

H55223 SOURCE human clone=C22\_204 primer=T3 library=Chromosome 22  
exon

Trofatter, et al., *Genome Res.* 5 (3): 214-224 (1995)

SEQ ID No. 15

1 CATGCTGGTG ATCCTGCTGA ACTGCGTGAC ACTTGGCATG TACCAGCCGT  
GCGACGACAT

61 GGA CTGCCTG TCCGACCGCT GCAAGATCCT GCAG

H55544 SOURCE human clone=C22\_651 primer=T3 library=Chromosome 22  
exon

- 11 -

Trofatter, et al. *Genome Res.* 5 (3): 214-224 (1995)

SEQ ID No. 16

1 GTATCTCTGG TTA CTTTAGT AGCCAACACT CTTGGCTACT CAGACCTTGG  
TCCCATTA  
61 TCCCTGCGAA CCTTGAGAGC ACTAAGACCT CTAAGAGCTT TGTCTAGATT  
TGAAGGAATG  
121 AGG

F07776 SOURCE human.

Submitted (19-JAN-1995) Genethon, B.P. 60, 91002 Evry Cedex France  
and Genetique Moleculaire et Biologie du developpement, CNRS UPR420  
B.P. 8, 94801 Villejuif Cedex France E-mail: genexpress@genethon.fr

SEQ ID No. 17

1 TTCTCTCCAT TGTAGGAATG TTTCTGGCTG AACTGATAGA AAAGTATTTT  
GTGTGCCCTA  
61 CCCTGTTNCG AGTGATCCGT CTTGCCAGGA TTGGCCGAAT CCTACGTCTG  
ATCAAAGGAG  
121 CAAAGGGGAT CCGCACGCTG CTCTTTGCTT TGATGATGTC CCTTCCTGCG  
TTGTTTAACA  
181 TCGGNCTCCT TCTTTTCCTG GTCATGTTCA TCTACGNCAT CTTTGGGATG  
TCCAATTTTG  
241 CCTATGTTAA GAGGGAAGTT GGGATCGATG ACATGTTNAN CTTTGAGACC  
TTTGGCAACA  
301 GCATGATCTG CCTGTTCCAA ATTACAACCT CTGCTGGCTG GGA

A search of the Sanger Genome Sequencing Center (Cambridge, U.K.) and the Washington University Genome Sequencing Center (St. Louis, MO) sequences in progress revealed a Bacterial Artificial Chromosome (BAC) sequence (bK206c7) that contained matches to the *C. elegans* cosmid open reading frame, C54D2.5, and to the four human chromosome 22 ESTs, H55225, H55617, H55223, H55544. The *C. elegans* C54D2.5 cosmid sequence and the human EST sequences were then used to compare the translation of the

- 12 -

bK206c7 BAC genomic sequence in all 6 reading frames. The analysis was performed using the graphical program Dotter (Eric Sohnhammer, NCBI). The analysis revealed a series of potential coding regions on one strand of the bK206c7 BAC sequence. These were subsequently translated in all 3 reading frames and the potential splice junctions identified. The translated sequence of this longer DNA fragment which is part of the human  $\alpha_{11}$  subunit gene is given by Seq. ID No. 18.

Using the sequence information from the five EST's, a full length gene can be recovered using any of several techniques. Polynucleotide probes having a sequence which corresponds to or hybridizes with the EST sequences or a distinctive portion thereof (for example oligonucleotide probes having a length of 18 to 100 nucleotides) can be used to probe a human cDNA library for identification of the full length DNA encoding the  $\alpha_{11}$  and  $\alpha_{1H}$  subunits. The process of identifying cDNAs of interest using defined probes is well known in the art and is, for example, described in International Patent Publication No. WO95/04144, which is incorporated herein by reference. This process generally involves screening bacterial hosts (e.g. *E. coli*) harboring the library plasmids or infected with recombinant lambda phage with labeled probes, e.g. radiolabeled with  $^{32}\text{P}$ , and selection of colonies or phage which bind the labeled probe. Each selected colony or phage is grown up, and the plasmids are recovered. Human cDNAs are recovered from the plasmids by restriction digestion, or can be amplified, for example by PCR. The recovered cDNA can be sequenced, and the position of the calcium channel subunit-encoding region further refined, although neither process is not necessary to the further use of the cDNA to produce cell lines expressing the novel calcium channel subunits.

Longer portions of DNA-encoding the novel calcium channel subunits of the invention can also be recovered by PCR cloning techniques using primers corresponding to or based upon the EST sequences. Using this technique to identify relevant sequences within a human brain total RNA preparation confirmed that the novel  $\alpha_{11}$  calcium channel subunit is present in human brain. Subcloning of the 567 nt PCR product and subsequent sequencing thereof showed that this product corresponds to the derived sequence from the bK206c7 BAC genomic sequence. The nucleotide sequence is given as SEQ ID No. 19. The same

- 13 -

experiment was performed using a rat brain RNA preparation and resulted in recovery of a substantially identical PCR product. (SEQ ID. NO. 20). The protein encoded by the rat PCR product is 96% identical to the human PCR product.

These sequences, which presumably encode a partial subunit can be used as a basis for constructing full length human or rat  $\alpha_{11}$  clones. Briefly, the subcloned  $\alpha_{11}$  PCR product is radiolabeled by random hexamer priming according to standard methods (See, Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor Press) and used to screen commercial human brain cDNA libraries (Stratagene, La Jolla, CA). The screening of cDNA libraries follows standard methods and includes such protocols as infecting bacteria with recombinant lambda phage, immobilizing lambda DNA to nitrocellulose filters and screening under medium hybridization stringency conditions with radiolabeled probe. cDNA clones homologous to the probe are identified by autoradiography. Positive clones are purified by sequential rounds of screening.

Following this protocol, most purified cDNA's are likely to be partial sequence clones due the nature of the cDNA library synthesis. Full length clones are constructed from cDNA's which overlap in DNA sequence. Restriction enzyme sites which overlap between cDNAs are used to ligate the individual cDNA's to generate a full-length cDNA. For subsequent heterologous expression, the full-length cDNA is subcloned directly into an appropriate vertebrate expression vector, such as pcDNA-3 (Invitrogen, San Diego, CA) in which expression of the cDNA is under the control of a promoter such as the CMV major intermediate early promoter/enhancer. Other suitable expression vectors include, for example, pMT2, pRC/CMV, pcDNA3.1 and pCEP4.

Once the full length cDNA is cloned into an expression vector, the vector is then transfected into a host cell for expression. Suitable host cells include *Xenopus* oocytes or mammalian cells such as human embryonic kidney cells as described in International Patent Publication No. WO 96/39512 which is incorporated herein by reference and Ltk cells as described in US Patent No. 5,386,025 which is incorporated herein by reference. Transfection into host cells may be accomplished by microinjection, lipofection, glycerol shock, electroporation calcium phosphate or particle-mediated gene transfer. The vector may also be

- 14 -

transfected into host cells to provide coexpression of the novel  $\alpha_1$  subunits with a  $\beta$  and/or an  $\alpha_2\delta$  subunit.

The resulting cell lines expressing functional calcium channels including the novel  $\alpha_1$  subunits of the invention can be used test compounds for pharmacological activity with respect to these calcium channels. Thus, the cell lines are useful for screening compounds for pharmaceutical utility. Such screening can be carried out using several available methods for evaluation of the interaction, if any, between the test compound and the calcium channel. One such method involves the binding of radiolabeled agents that interact with the calcium channel and subsequent analysis of equilibrium binding measurements including but not limited to, on rates, off rates,  $K_d$  values and competitive binding by other molecules. Another such method involves the screening for the effects of compounds by electrophysiological assay whereby individual cells are impaled with a microelectrode and currents through the calcium channel are recorded before and after application of the compound of interest. Another method, high-throughput spectrophotometric assay, utilizes the loading the cell lines with a fluorescent dye sensitive to intracellular calcium concentration and subsequent examination of the effects of compounds on the ability of depolarization by potassium chloride or other means to alter intracellular calcium levels. Compounds to be tested as agonists or antagonists of the novel  $\alpha_{11}$  and  $\alpha_{1H}$  calcium channel subunits are combined with cells that are stably or transiently transformed with a DNA sequence encoding the  $\alpha_{11}$  or  $\alpha_{1H}$  calcium channel subunits of the invention and monitored using one of these techniques.

DNA fragments with sequences given by SEQ ID Nos. 13-19 may also be used for mapping the distribution of  $\alpha_{11}$  and  $\alpha_{1H}$  calcium channel subunits within a tissue sample. This method follows normal histological procedures using a nucleic acid probe, and generally involves the steps of exposing the tissue to a reagent comprising a directly or indirectly detectable label coupled to a selected DNA fragment, and detecting reagent that has bound to the tissue. Suitable labels include fluorescent labels, enzyme labels, chromophores and radio-labels.



- 15 -

EXAMPLE 1

In order to isolate novel human calcium channel  $\alpha_1$  subunits using standard molecular cloning protocols, synthetic DNA probes are prepared, radiolabeled with  $^{32}\text{P}$  and utilized to screen human cDNA libraries commercially available in lambda phage vectors (Stratagene, La Jolla, CA) based on the human DNA sequences for H55225, H55617, H55223, H55544 and F07776. DNA fragments with the sequence of sequence ID NOs 18 and 19 may also be used for this purpose. Positive phage are purified through several rounds of screening involving immobilizing the phage DNA on nitrocellulose filters, hybridizing with the radiolabeled probe, washing off of excess probe and then selection of clones by autoradiography. Clones identified by this approach are expected to be partial length clones due to the nature of cDNA library synthesis and several rounds of screening for each calcium channel type may be necessary to obtain full-length clones.

To characterize the clones, double stranded plasmid DNA is prepared from the identified clones and the sequences are determined using  $^{35}\text{S}$  dATP, Sequenase and standard gel electrophoresis methods. Regions of similarity and regions of overlap are determined by comparison of each cDNA sequence.

Full-length clones are constructed by ligating overlapping cDNA fragments together at common restriction enzyme sites. The full-length clones are subsequently inserted into vectors suitable for expression in vertebrate cells (e.g. pMT2, pRC/CMV, pcDNA3.1, pCEP4, pREP7) by ligation into restriction sites in the vector polylinker region which is downstream of the promoter used to direct cDNA expression.

DNA encoding the novel calcium channels can be stably or transiently introduced into eukaryotic cells (e.g. human embryonic kidney, mouse L cells, chinese hamster ovary, etc) by any number of available standard methods. Stable transfection is achieved by growing the cells under conditions that promote growth of cells expressing a marker gene which is contained in the expression vector (e.g. dihydrofolate reductase, thymidine kinase, or the like). The heterologous DNA encoding the human calcium channel may be integrated into the genome or may be maintained as an episomal element.

- 16 -

Expression of the human calcium channel in transfected cells may monitored by any number of techniques, including Northern blot for RNA analysis, Southern blot for cDNA detection, electrophysiological assay for calcium channel function, the binding of radiolabeled agents thought to interact with the calcium channel, and fluorescent assay of dyes sensitive to intracellular calcium concentration.

## EXAMPLE 2

### Heterologous Expression of Human $\alpha_{11}$ Calcium Channels in Cells

#### A. Transient Transfection in Mammalian Cells

Host cells, such as human embryonic kidney cells, HEK 293 (ATCC# CRL 1573) are grown in standard DMEM medium supplemented with 2 mM glutamine and 10% fetal bovine serum. HEK 293 cells are transfected by a standard calcium-phosphate-DNA co-precipitation method using the full-length human  $\alpha_{11}$  calcium channel cDNA in a vertebrate expression vector (for example see Current protocols in Molecular Biology). The human  $\alpha_{11}$  calcium channel cDNA may be transfected alone or in combination with other cloned subunits for mammalian calcium channels, such as  $\alpha_{2\delta}$  and  $\beta$  subunits, and also with clones for marker proteins such the jellyfish green fluorescent protein.

Electrophysiological Recording: After an incubation period of from 24 to 72 hrs the culture medium is removed and replaced with external recording solution (see below). Whole cell patch clamp experiments are performed using an Axopatch 200B amplifier (Axon Instruments, Burlingame, CA) linked to an IBM compatible personal computer equipped with pCLAMP software. Microelectrodes are filled with 3 M CsCl and have typical resistances from 0.5 to 2.5 M $\Omega$ . The external recording solution is 20 mM BaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM HEPES, 40 mM TEACl, 10 mM Glucose, 65 mM CsCl, (pH 7.2). The internal pipette solution is 105 mM CsCl, 25 mM TEACl, 1 mM CaCl<sub>2</sub>, 11 mM EGTA, 10 mM HEPES (pH 7.2). Currents are typically elicited from a holding potential of -100 mV to various test potentials. Data are filtered at 1 kHz and recorded directly on the harddrive of a personal computer. Leak subtraction is carried out on-line using a standard P/5 protocol. Currents are

- 17 -

analyzed using pCLAMP versions 5.5 and 6.0. Macroscopic current-voltage relations are fitted with the equation  $I = \{1/(1+\exp(-(V_m-V_h)/S))\} \times G - (V_m-E_{rev})$ , where  $V_m$  is the test potential,  $V_h$  is the voltage at which half of the channels are activated, and  $S$  reflects the steepness of the activation curve and is an indication of the effective gating charge movement. Inactivation curves are normalized to 1 and fitted with  $I = (1/1 + \exp((V_m-V_h)/S))$  with  $V_m$  being the holding potential. Single channel recordings are performed in the cell-attached mode with the following pipette solution (in mM): 100 BaCl<sub>2</sub>, 10 HEPES, pH 7.4 and bath solution: 100 KCl, 10 EGTA, 2 MgCl<sub>2</sub>, 10 HEPES, pH 7.4.

#### **B. Transient Transfection in Xenopus Oocytes**

Stage V and VI Xenopus oocytes are prepared as described by Dascal et al (1986), Expression and modulation of voltage-gated calcium channels after RNA injection into Xenopus oocytes. Science 231:1147-1150. After enzymatic dissociation with collagenase, oocytes nuclei are microinjected with the human  $\alpha_{11}$  calcium channel cDNA expression vector construct (approximately 10 ng DNA per nucleus) using a Drummond nanoject apparatus. The human  $\alpha_{11}$  calcium channel may be injected alone, or in combination with other mammalian calcium channel subunit cDNAs, such as the  $\alpha_2\text{-}\delta$  and  $\beta_1b$  subunits. After incubation from 48 to 96 hrs macroscopic currents are recorded using a standard two microelectrode voltage-clamp (Axoclamp 2A, Axon Instruments, Burlingame, CA) in a bathing medium containing (in mM): 40 Ba(OH)<sub>2</sub>, 25 TEA-OH, 25 NaOH, 2 CsOH, 5 HEPES (pH titrated to 7.3 with methan-sulfonic acid). Pipettes of typical resistance ranging from 0.5 to 1.5 M $\Omega$  are filled with 2.8M CsCl, 0.2M CsOH, 10mM HEPES, 10mM BAPTA free acid. Endogenous Ca (and Ba) -activated Cl currents are suppressed by systematically injecting 10-30 nl of a solution containing 100mM BAPTA-free acid, 10mM HEPES (pH titrated to 7.2 with CsOH) using a third pipette connected to a pneumatic injector. Leak currents and capacitive transients are subtracted using a standard P/5 procedure.

- 18 -

EXAMPLE 3Construction of Stable Cell Lines Expressing Human  $\alpha_{11}$  Calcium Channels

Mammalian cell lines stably expressing human  $\alpha_{11}$  calcium channels are constructed by transfecting the  $\alpha_{11}$  calcium channel cDNA into mammalian cells such as HEK 293 and selecting for antibiotic resistance encoded for by an expression vector. Briefly, the full-length human  $\alpha_{11}$  calcium channel cDNA subcloned into a vertebrate expression vector with a selectable marker, such as the pcDNA3 (InvitroGen, San Diego, CA), is transfected into HEK 293 cells by calcium phosphate coprecipitation or lipofection or electroporation or other method according to well known procedures (Methods in Enzymology, Volume 185, Gene Expression Technology (1990) Edited by Goeddel, D.V.). The human  $\alpha_{11}$  calcium channel may be transfected alone, or in combination with other mammalian calcium channel subunit cDNAs, such as the  $\alpha_2$ - $\delta$  and  $\beta_1$  subunits, either in a similar expression vector or other type of vector using different selectable markers. After incubation for 2 days in nonselective conditions, the medium is supplemented with Geneticin (G418) at a concentration of between 600 to 800 ug/ml. After 3 to 4 weeks in this medium, cells which are resistant to G418 are visible and can be cloned as isolated colonies using standard cloning rings. After growing up each isolated colony to confluency to establish cell lines, the expression of human  $\alpha_{11}$  calcium channels can be determined at with standard gene expression methods such as Northern blotting, RNase protection and reverse-transcriptase PCR.

The functional detection of human  $\alpha_{11}$  calcium channels in stably transfected cells can be examined electrophysiologically, such as by whole patch clamp or single channel analysis (see above). Other means of detecting functional calcium channels include the use of radiolabeled  $^{45}\text{Ca}$  uptake, fluorescence spectroscopy using calcium sensitive dyes such as FURA-2, and the binding or displacement of radiolabeled ligands that interact with the calcium channel.

**SEQUENCE LISTING**

(1) GENERAL INFORMATION:

(i) APPLICANT: Snutch, Terry P.  
Baillie, David L.

- 19 -

(ii) TITLE OF INVENTION: Novel Human Calcium Channels and Related Probes, Cell Lines and Methods

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE:

(B) STREET:

(C) CITY:

(D) STATE:

(E) COUNTRY:

(F) ZIP:

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette, 3.50 inch, 1.44 Kb storage

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: MS DOS 6.0

(D) SOFTWARE: WordPerfect

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

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(C) REFERENCE/DOCKET NUMBER: NMED.P-001-US

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTCAAAACTC AGGCCTTCTA CTGG 24

- 20 -

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:  
AACGTGTTCT TGGCTATCGC GGTG 24

## (2) INFORMATION FOR SEQ ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:  
GTGAAAGCAC AGAGCTTCTA CTGG 24

## (2) INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

- 21 -

AACGTTTTCT TGGCCATTGC TGTG 24

## (2) INFORMATION FOR SEQ ID NO: 5:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GTAAATCCA ACGTCTTCTA CTGG 28

## (2) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AATGTGTTCT TGGCCATTGC GGTG 24

## (2) INFORMATION FOR SEQ ID NO: 7:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

- 22 -

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:  
GTGAAGTCTG TCACGTTTCTA CTGG 24

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:  
AAGCTCTTCT TGGCCATTGC TGTA 24

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:  
GTCAAGTCGC AAGTGTTCTA CTGG 24

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no



- 23 -

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

AATGTATTCT TGGCTATCGC TGTG 24

## (2) INFORMATION FOR SEQ ID NO: 11:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ATCTAYGCRY TSATYGGSAT G 21

## (2) INFORMATION FOR SEQ ID NO: 12:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

ATGGACAA YT TYGASTAYTC 20

## (2) INFORMATION FOR SEQ ID NO: 13:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 168

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- 24 -

- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: human
- (ix) FEATURE: expressed sequence tag H55225
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

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GTGATCACTC TGAAGGCTG GGTGGAGATC ATGTACTACG TGATGGATGC TCACTCCTTC   60
TACAACTTCA TCTACTTCAT CCTGCTTATC ATACCCCTCT TGCCTTGAC CCGATATGGT  120
CTTCCCAGAG TGAGCTCATC CACCTCGTCA TGCCTGACTC GACGTTCA                168

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## (2) INFORMATION FOR SEQ ID NO: 14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: human
- (ix) FEATURE: expressed sequence tag H55617
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```

GATGGTCGAG TACTCCCTGG ACCTTCAGAA CATCAACCTG TCAGCCATCC GCACCGTGCG   60
CGTCTGAGG CCCCTCAAAG CCATCAACCG CGTGCCCA                98

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## (2) INFORMATION FOR SEQ ID NO: 15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 94
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (iii) HYPOTHETICAL: no
- (iv) ANTI-SENSE: no
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: human
- (ix) FEATURE: expressed sequence tag H55223
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```

CATGCTGGTG ATCCTGCTGA ACTGCGTGAC ACTTGGCATG TACCAGCCGT GCGACGACAT   60
GGACTGCCTG TCCGACCGCT GCAAGATCCT GCAG                                94

```

- 25 -

## (2) INFORMATION FOR SEQ ID NO: 16:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 123

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

(ix) FEATURE: expressed sequence tag H55544

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GTATCTCTGG	TTACTTTAGT	AGCCAACACT	CTTGGCTACT	CAGACCTTGG	TCCCATTAAA	60
TCCCTGCGAA	CCTTGAGAGC	ACTAAGACCT	CTAAGAGCTT	TGTCTAGATT	TGAAGGAATG	120
AGG						123

## (2) INFORMATION FOR SEQ ID NO: 17:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 343

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

(ix) FEATURE: expressed sequence tag F07776

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

TTCTCTCCAT	TGTAGGAATG	TTTCTGGCTG	AACTGATAGA	AAAGTATTTT	GTGTGCCCTA	60
CCCTGTTNCG	AGTGATCCGT	CTTGCCAGGA	TTGGCCGAAT	CCTACGTCTG	ATCAAAGGAG	120
CAAAGGGGAT	CCGCACGCTG	CTCTTTGCTT	TGATGATGTC	CCTTCCTGCG	TTGTTTAACA	180
TCGGNCTCCT	TCTTTTCCTG	GTCATGTTCA	TCTACGNCAT	CTTTGGGATG	TCCAATTTTG	240
CCTATGTTAA	GAGGGAAGTT	GGGATCGATG	ACATGTTNAN	CTTTGAGACC	TTTGGCAACA	300
GCATGATCTG	CCTGTTCCAA	ATTACAACCT	CTGCTGGCTG	GGA		343

## (2) INFORMATION FOR SEQ ID NO: 18:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5562

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

- 26 -

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

(ix) FEATURE: human alpha-I partial sequence from BAC bK206c7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

ATG TTT TTC GTC TCA GCC AAT CCC TGG GTG AGT TTC ACC AGT TTT GAT TTA AAC	54
Met Phe Phe Val Ser Ala Asn Pro Trp Val Ser Phe Thr Ser Phe Asp Leu Asn	
GTG GCC AAT ATG GAC AAC TTC TTC GCC CCC GTT TTC ACC ATG GGC AAA TAT TAT	108
Val Ala Asn Met Asp Asn Phe Phe Ala Pro Val Phe Thr Met Gly Lys Tyr Tyr	
ACG CAA GGC GAC AAG GTG CTG ATG CCG CTG GCG ATT CAG GCT CTG AAA CAG CTG	162
Thr Gln Gly Asp Lys Val Leu Met Pro Leu Ala Ile Gln Ala Leu Lys Gln Leu	
ATG TTC AAA TTG GTG GCC ACT GTT GCT CGA ACA CAT GCT ACA CCG TCA CAC ATC	206
Met Phe Lys Leu Val Ala Thr Val Ala Arg Thr His Ala Thr Pro Ser His Ile	
ACG GGT GGT CCT GGA ACA GGG ATG CAC ACG GGC ACC TTC CAG GAA GGA GCT GAG	270
Thr Gly Gly Pro Gly Thr Gly Met His Thr Gly Thr Phe Gln Glu Gly Ala Glu	
CCT GGT TCA TCT CAG CAC CCT GAG GCA CAG GCC ACG TAT ACA GCA GGG TGC ACC	324
Pro Gly Ser Ser Gln His Pro Glu Ala Gln Ala Thr Tyr Thr Ala Gly Cys Thr	
CCA GCC CCC ACG GGC GAT CCC ACC TGC TGC TTT GTC CTT GAC TTG GTG TGC ACG	378
Pro Ala Pro Thr Gly Asp Pro Thr Cys Cys Phe Val Leu Asp Leu Val Cys Thr	
TGG TTT GAA TGT GTC AGC ATG CTG GTG ATC CTG CTG AAC TGC GTG ACA CTT GGC	432
Trp Phe Glu Cys Val Ser Met Leu Val Ile Leu Leu Asn Cys Val Thr Leu Gly	
ATG TAC CAG CCG TGC GAC GAC ATG GAC TGC CTG TCC GAC CGC TGC AAG ATC CTG	486
Met Tyr Gln Pro Cys Asp Asp Met Asp Cys Leu Ser Asp Arg Cys Lys Ile Leu	
CAG GTC TTT GAT GAC TTC ATC TTT ATC TTC TTT GCC ATG GAG ATG GTG CTC AAG	540
Gln Val Phe Asp Asp Phe Ile Phe Ile Phe Phe Ala Met Glu Met Val Leu Lys	
ATG GTG GCC CTG GGG ATT TTT GGC AAG AAG TGC TAC CTC GGG GAC ACA TGG AAC	594
Met Val Ala Leu Gly Ile Phe Gly Lys Lys Cys Tyr Leu Gly Asp Thr Trp Asn	

- 27 -

CGC CTG GAT TTC TTC ATC GTC ATG GCA GGC AAC ATC AAC CTG TCA GCC ATC CGC Arg Leu Asp Phe Phe Ile Val Met Ala Gly Asn Ile Asn Leu Ser Ala Ile Arg	648
ACC GTG CGC GTC CTG AGG CCC CTC AAA GCC ATC AAC CGC GTG CCC AGT ATG CGG Thr Val Arg Val Leu Arg Pro Leu Lys Ala Ile Asn Arg Val Pro Ser Met Arg	702
ATC CTG GTG AAC CTG CTC CTG GAC ACA CTG CCC ATG CTG GGG AAT GTC CTG CTG Ile Leu Val Asn Leu Leu Leu Asp Thr Leu Pro Met Leu Gly Asn Val Leu Leu	756
CTC TGC TTC TTT GTC TTC TTC ATC TTT GGC ATC ATA GGT GTG CAG CTC TGG GCG Leu Cys Phe Phe Val Phe Phe Ile Phe Gly Ile Ile Gly Val Gln Leu Trp Ala	810
GGC CTG CTG CGT AAC CGC TGC TTC CTG GAG GAG AAC TTC ACC ATA CAA GGG GAT Gly Leu Leu Arg Asn Arg Cys Phe Leu Glu Glu Asn Phe Thr Ile Gln Gly Asp	864
GTG GCC TTG CCC CCA TAC TAC CAG CCG GAG GAG GAT GAT GAG ATG CCC TTC ATC Val Ala Leu Pro Pro Tyr Tyr Gln Pro Glu Glu Asp Asp Glu Met Pro Phe Ile	918
TGC TCC CTG TCG GGC GAC AAT GGG ATA ATG GGC TGC CAT GAG ATC CCC CCG CTC Cys Ser Leu Ser Gly Asp Asn Gly Ile Met Gly Cys His Glu Ile Pro Pro Leu	972
AAG GAG CAG GGC CGT GAG TGC TGC CTG TCC AAG GAC GAC GTC TAC GAC TTT GGG Lys Glu Gln Gly Arg Glu Cys Cys Leu Ser Lys Asp Asp Val Tyr Asp Phe Gly	1026
GCG GGG CGC CAG GAC CTC AAT GCC AGC GGC CTC TGT GTC AAC TGG AAC CGT TAC Ala Gly Arg Gln Asp Leu Asn Ala Ser Gly Leu Cys Val Asn Trp Asn Arg Tyr	1080
TAC AAT GTG TGC CGC ACG GGC AGC GCC AAC CCC CAC AAG GGT GCC ATC AAC TTT Tyr Asn Val Cys Arg Thr Gly Ser Ala Asn Pro His Lys Gly Ala Ile Asn Phe	1134
GAC AAC ATC GGT TAT GCT TGG ATT GTC ATC TTC CAG GTG ATC ACT CTG GAA GGC Asp Asn Ile Gly Tyr Ala Trp Ile Val Ile Phe Gln Val Ile Thr Leu Glu Gly	1188
TGG GTG GAG ATC ATG TAC TAC GTG ATG GAT GCT CAC TCC TTC TAC AAC TTC ATC Trp Val Glu Ile Met Tyr Tyr Val Met Asp Ala His Ser Phe Tyr Asn Phe Ile	1242
TAC TTC ATC CTG CTT ATC ATA AGT GAG CTC ATC CAC CTC GTC ATG CCT GAC TGC Tyr Phe Ile Leu Leu Ile Ile Ser Glu Leu Ile His Leu Val Met Pro Asp Cys	1296
AGC TTC AGC ACA GCA CAG TCC CCA AAA TGT CAA GGT GAT TCA CTC CCA GGA GTC Ser Phe Ser Thr Ala Gln Ser Pro Lys Cys Gln Gly Asp Ser Leu Pro Gly Val	1350

- 28 -

GCT GCT GAA TCC CTG CTG CTG CGA GAC TCT AGC TCC TCA GTC ATC ACT GAT GAG 1404  
Ala Ala Glu Ser Leu Leu Leu Arg Asp Ser Ser Ser Ser Val Ile Thr Asp Glu

GCT GCA GCC ATG GAG AAC CTC CTG GCG GGC ACC TCC AAG GGG GAT GAA AGC TAT 1458  
Ala Ala Ala Met Glu Asn Leu Leu Ala Gly Thr Ser Lys Gly Asp Glu Ser Tyr

CTG CTC AGG CTG GCC GGC AGC CAA GTT CAC TCC CAG GCT CAG CAA ATG CTG GGG 1512  
Leu Leu Arg Leu Ala Gly Ser Gln Val His Ser Gln Ala Gln Gln Met Leu Gly

AGG GGG CTG GGC CCT GAA AGC CTG GAA ACT GGA GAG GAG CCC CAC TCG TGG AGC 1566  
Arg Gly Leu Gly Pro Glu Ser Leu Glu Thr Gly Glu Glu Pro His Ser Trp Ser

CCT CGG GCC ACA AGG AGA TGG GAT CCC CAA TGC CAA CCA GGG CAG CCT CTC CCC 1620  
Pro Arg Ala Thr Arg Arg Trp Asp Pro Gln Cys Gln Pro Gly Gln Pro Leu Pro

CTT CAT TTC ATG CAA GCA CAG GTG GGC TCC TTC TTC ATG ATC AAC CTG TGC CTC 1674  
Leu His Phe Met Gln Ala Gln Val Gly Ser Phe Phe Met Ile Asn Leu Cys Leu

GTT GTC ATA GCG ACC CAG TTC TCG GAG ACC AAG CAA CGG GAG CAC CGG CTG ATG 1728  
Val Val Ile Ala Thr Gln Phe Ser Glu Thr Lys Gln Arg Glu His Arg Leu Met

CTG GAG CAG CGG CAG CGC TAC CTG TCC TCC AGC ACG GTG GCC AGC TAC GCC GAG 1782  
Leu Glu Gln Arg Gln Arg Tyr Leu Ser Ser Ser Thr Val Ala Ser Tyr Ala Glu

CCT GGC GAC TGC TAC GAG GAG ATC TTC CAG TAT GTC TGC CAC ATC CTG CGC AAG 1836  
Pro Gly Asp Cys Tyr Glu Glu Ile Phe Gln Tyr Val Cys His Ile Leu Arg Lys

GCC AAG CGC CGC GCC CTG GGC CTC TAC CAG GCC CTG CAG AGC CGG CGC CAG GCC 1890  
Ala Lys Arg Arg Ala Leu Gly Leu Tyr Gln Ala Leu Gln Ser Arg Arg Gln Ala

CTG GGC CCG GAG GCC CCG GCC CCC GCC AAA CCT GGG CCC CAC GCC AAG GAG CCC 1944  
Leu Gly Pro Glu Ala Pro Ala Pro Ala Lys Pro Gly Pro His Ala Lys Glu Pro

CGG CAC TAC CCT CTC ACA GTC TGG GAA TCG ATT CTT GGG AGG CAA GCA GAA GAA 1998  
Arg His Tyr Pro Leu Thr Val Trp Glu Ser Ile Leu Gly Arg Gln Ala Glu Glu

TGC ACG CTC AGA GCT GCC GCC CAC CCG TCC TCG GGT GCC AGC CAT CCA GGC GTG 2049  
Cys Thr Leu Arg Ala Ala Ala His Pro Ser Ser Gly Ala Ser His Pro Gly Val

SUBSTITUTE SHEET (RULE 26)

- 29 -

GGC TCG GAG GAG GCC CCA GAG CTG TGC CCG CAA CAT AGC CCC CTG GAT GCG ACG 2106  
 Gly Ser Glu Glu Ala Pro Glu Leu Cys Pro Gln His Ser Pro Leu Asp Ala Thr

CCC CAC ACC CTG GTG CAG CCC ATC CCC GCC ACG CTG GCT TCC GAT CCC GCC AGC 2160  
 Pro His Thr Leu Val Gln Pro Ile Pro Ala Thr Leu Ala Ser Asp Pro Ala Ser

TGC CCT TGC TGC CAG CAT GAG GAC GGC CGG CGG CCC TCG GGC CTG GGC AGC ACC 2214  
 Cys Pro Cys Cys Gln His Glu Asp Gly Arg Arg Pro Ser Gly Leu Gly Ser Thr

GAC TCG GGC CAG GAG GGC TCG GGC TCC GGG AGC TCC GCT GGT GGC GAG GAC GAG 2268  
 Asp Ser Gly Gln Glu Gly Ser Gly Ser Gly Ser Ser Ala Gly Gly Glu Asp Glu

GCG GAT GGG GAC GGG GCC CGG AGC AGC GAG GAC GGA GCC TCC TCA GAA CTG GGG 2322  
 Ala Asp Gly Asp Gly Ala Arg Ser Ser Glu Asp Gly Ala Ser Ser Glu Leu Gly

AAG GAG GAG GAG GAG GAG GAG CAG GCG GAT GGG GCG GTC TGG CTG TGC GGG GAT 2376  
 Lys Glu Glu Glu Glu Glu Glu Gln Ala Asp Gly Ala Val Trp Leu Cys Gly Asp

GTG TGG CGG GAG ACG CGA GCC AAG CTG CGC GGC ATC GTG GAC AGC AAG TAC TTC 2430  
 Val Trp Arg Glu Thr Arg Ala Lys Leu Arg Gly Ile Val Asp Ser Lys Tyr Phe

AAC CGG GGC ATC ATG ATG GCC ATC CTG GTC AAC ACC GTC AGC ATG GGC ATC GAG 2484  
 Asn Arg Gly Ile Met Met Ala Ile Leu Val Asn Thr Val Ser Met Gly Ile Glu

CAC CAC GAG CAG GCC AGT GCA GCG CAG CCG GGC CGG GCC TGC GGG AGA GGA CAA 2538  
 His His Glu Gln Ala Ser Ala Ala Gln Pro Gly Arg Ala Cys Gly Arg Gly Gln

AAT CCA GAC CTT TGC ATG ACC CTC AAG GCC CCT TGT CTC TGT CAC AAC GTC CCT 2592  
 Asn Pro Asp Leu Cys Met Thr Leu Lys Ala Pro Cys Leu Cys His Asn Val Pro

TCA CCA GGC CAG GGT GTC CTG TCC CAT CCA GTG ACT CCA CCC CAT ACA GCC CCA 2646  
 Ser Pro Gly Gln Gly Val Leu Ser His Pro Val Thr Pro Pro His Thr Ala Pro

TGG CGC ATG GAG ACA GGA AAG CAG GGA CAC GGA TGT GAA GAA GGA CCA GGA CAA 2700  
 Trp Arg Met Glu Thr Gly Lys Gln Gly His Gly Cys Glu Glu Gly Pro Gly Gln

CGA AGC AGT GAC ATG TTT GCC CTG GAG ATG ATC CTG AAG CTG GCT GCA TTT GGG 2754  
 Arg Ser Ser Asp Met Phe Ala Leu Glu Met Ile Leu Lys Leu Ala Ala Phe Gly

- 30 -

CTC TTC GAC TAC CTG CGT AAC CCC TAC AAC ATC TTC GAC AGC ATC ATT GTC ATC 2808  
Leu Phe Asp Tyr Leu Arg Asn Pro Tyr Asn Ile Phe Asp Ser Ile Ile Val Ile

ATC AGC ATC TGG GAG ATC GTG GGG CAG GCG GAC GGT GGG CTG TCG GTG CTG CGG 2862  
Ile Ser Ile Trp Glu Ile Val Gly Gln Ala Asp Gly Gly Leu Ser Val Leu Arg

ACC TTC CGG CTG CTG CGC GTG CTG AAA CTG GTG CGC TTC ATG CCT GCC CTG CGG 2916  
Thr Phe Arg Leu Leu Arg Val Leu Lys Leu Val Arg Phe Met Pro Ala Leu Arg

CGC CAG CTC GTG GTG CTC ATG AAG ACC ATG GAC AAC GTG GCC ACC TTC TGC ATG 2970  
Arg Gln Leu Val Val Leu Met Lys Thr Met Asp Asn Val Ala Thr Phe Cys Met

CTG CTC ATG CTC TTC ATC TTC ATC TTC AGC ATC CTT GGG ATG CAT ATT TTT GGC 3024  
Leu Leu Met Leu Phe Ile Phe Ile Phe Ser Ile Leu Gly Met His Ile Phe Gly

TGC AAG TTC AGC CTC CGC ACG GAC ACT GGA GAC ACG GTG CCC GAC AGG AAG AAC 3078  
Cys Lys Phe Ser Leu Arg Thr Asp Thr Gly Asp Thr Val Pro Asp Arg Lys Asn

TTC GAC TCC CTG CTG TGG GCC ATC GTC ACT GTG TTC CAG ATC CTC ACC CAG GAG 3132  
Phe Asp Ser Leu Leu Trp Ala Ile Val Thr Val Phe Gln Ile Leu Thr Gln Glu

GAC TGG AAC GTC GTT CTC TAC AAT GGC ATG GCC TCC ACT TCT CCC TGG GCC TCC 3186  
Asp Trp Asn Val Val Leu Tyr Asn Gly Met Ala Ser Thr Ser Pro Trp Ala Ser

CTC TAC TTT GTC GCC CTC ATG ACC TTC GGC AAC TAT GTG CTC TTC AAC CTG CTG 3240  
Leu Tyr Phe Val Ala Leu Met Thr Phe Gly Asn Tyr Val Leu Phe Asn Leu Leu

GTG GCC ATC CTG GTG GAG GGC TTC CAG GCG GAG GTG ACT GTG GTC TTG GCA GAG 3294  
Val Ala Ile Leu Val Glu Gly Phe Gln Ala Glu Val Thr Val Val Leu Ala Glu

GAA GCA CCC CCA CAG GGC CTG CGA AAG ACT GGG CGA GGG AGA GGT GGC CTG GAT 3348  
Glu Ala Pro Pro Gln Gly Leu Arg Lys Thr Gly Arg Gly Arg Gly Gly Leu Asp

GGG GGA GGG CTG CAA TTC AAA CTT CTA GCA GGC AAC CTA TCC CTA AAG GAG GGG 3402  
Gly Gly Gly Leu Gln Phe Lys Leu Leu Ala Gly Asn Leu Ser Leu Lys Glu Gly

GTT GCT GAT GAG GTG GGT GAC GCC AAT CGC TCC TAC TCG GAC GAG GAC CAG AGC 3456  
Val Ala Asp Glu Val Gly Asp Ala Asn Arg Ser Tyr Ser Asp Glu Asp Gln Ser

TCA TCC AAC ATA GAA GAG TTT GAT AAG CTC CAG GAA GGC CTG GAC AGC AGC GGA 3510  
Ser Ser Asn Ile Glu Glu Phe Asp Lys Leu Gln Glu Gly Leu Asp Ser Ser Gly

SUBSTITUTE SHEET (RULE 26)



- 31 -

GAT CCC AAG CTC TGC CCA ATC CCC ATG ACC CCC AAT GGG CAC CTG GAC CCC AGT 3564  
Asp Pro Lys Leu Cys Pro Ile Pro Met Thr Pro Asn Gly His Leu Asp Pro Ser

CTC CCA CTG GGT GGG CAC CTA GGT CCT GCT GGG GCT GCG GGA CCT GCC CCC CGA 3618  
Leu Pro Leu Gly Gly His Leu Gly Pro Ala Gly Ala Ala Gly Pro Ala Pro Arg

CTC TCA CTG CAG CCG GAC CCC ATG CTG GTG GCC CTG GGC TCC CGA AAG AGC AGC 3672  
Leu Ser Leu Gln Pro Asp Pro Met Leu Val Ala Leu Gly Ser Arg Lys Ser Ser

GTC ATG TCT CTA GGG AGG ATG AGC TAT GAC CAG CGC TCC CTG GTG GGT GGT CTT 3726  
Val Met Ser Leu Gly Arg Met Ser Tyr Asp Gln Arg Ser Leu Val Gly Gly Leu

AGA GCC ACA GCG GGG GTG CAG GCT GCC TTT GGG CAC CTG GTG CCC CAG CCG TGG 3780  
Arg Ala Thr Ala Gly Val Gln Ala Ala Phe Gly His Leu Val Pro Gln Pro Trp

GTG TGC CTG TGG GGC GCT GAC CCG AAC GGG AAC TCC TTC CAG TCC AGC TCC CGG 3834  
Val Cys Leu Trp Gly Ala Asp Pro Asn Gly Asn Ser Phe Gln Ser Ser Ser Arg

AGC TCC TAC TAC GGG CCA TGG GGC CGC AGC GCG GCC TGG GCC AGC CGT CGC TCC 3888  
Ser Ser Tyr Tyr Gly Pro Trp Gly Arg Ser Ala Ala Trp Ala Ser Arg Arg Ser

AGC TGG AAC AGC CTC AAG CAC AAG CCG CCG TCG GCG GAG CAT GAG TCC CTG CTC 3942  
Ser Trp Asn Ser Leu Lys His Lys Pro Pro Ser Ala Glu His Glu Ser Leu Leu

TCT GCG GAG CGC GGC GGC GGC GCC CGG GTC TGC GAG GTT GCC GCG GAC GAG GGG 3996  
Ser Ala Glu Arg Gly Gly Gly Ala Arg Val Cys Glu Val Ala Ala Asp Glu Gly

CCG CCG CCG GCC GCA CCC CTG CAC ACC CCA CAC GCC CAC CAC GTT CAT CAC GGG 4050  
Pro Pro Arg Ala Ala Pro Leu His Thr Pro His Ala His His Val His His Gly

CCC CAT CTG GCG CAC CGC CAC CGC CAC CAC CGC CGG ACG CTG TCC CTC GAC AAC 4104  
Pro His Leu Ala His Arg His Arg His His Arg Arg Thr Leu Ser Leu Asp Asn

AGG GAC TCG GTG GAC CTG GCC GAG CTG GTG CCC GCG GTG GGC GCC CAC CCC CGG 4158  
Arg Asp Ser Val Asp Leu Ala Glu Leu Val Pro Ala Val Gly Ala His Pro Arg

GCC GCC TGG AGG GCG GCA GGC CCG GCC CCC GGG CAT GAG GAC TGC AAT GGC AGG 4212  
Ala Ala Trp Arg Ala Ala Gly Pro Ala Pro Gly His Glu Asp Cys Asn Gly Arg

- 32 -

ATG CCC AGC ATC GCC AAA GAC GTC TTC ACC AAG ATG GGC GAC CGC GGG GAT CGC 4266  
 Met Pro Ser Ile Ala Lys Asp Val Phe Thr Lys Met Gly Asp Arg Gly Asp Arg

GGG GAG GAT GAG GAG GAA ATC GAC TAC GTG AGT GGG GGC GGG GCC GAA GGG GAC 4320  
 Gly Glu Asp Glu Glu Glu Ile Asp Tyr Val Ser Gly Gly Gly Ala Glu Gly Asp

CTG ACC CTG TGC TTC CGC GTC CGC AAG ATG ATC GAC GTC TAT AAG CCC GAC TGG 4374  
 Leu Thr Leu Cys Phe Arg Val Arg Lys Met Ile Asp Val Tyr Lys Pro Asp Trp

TGC GAG GTC CGC GAA GAC TGG TCT GTC TAC CTC TTC TCT CCC GAG AAC AGG CTC 4428  
 Cys Glu Val Arg Glu Asp Trp Ser Val Tyr Leu Phe Ser Pro Glu Asn Arg Leu

AGG GAT CTG GGC TGG GTA AGC CTC GAG TGC CAG GGA AAG GTG GGT GAC CTC GTG 4482  
 Arg Asp Leu Gly Trp Val Ser Leu Glu Cys Gln Gly Lys Val Gly Asp Leu Val

GTG TGG GTG TAT GGT CAG AGG AGG CAG CGC CAG ACC ATT ATT GCC CAC AAA CTC 4536  
 Val Trp Val Tyr Gly Gln Arg Arg Gln Arg Gln Thr Ile Ile Ala His Lys Leu

TTC GAC TAC GTC GTC CTG GCC TTC ATC TTT CTC AAC TGC ATC ACC ATC GCC CTG 4590  
 Phe Asp Tyr Val Val Leu Ala Phe Ile Phe Leu Asn Cys Ile Thr Ile Ala Leu

GAG CGG CCT CAG ATC GAG GCC GGC AGC ACC GAA CGC ATC TTT CTC ACC GTG TCC 4644  
 Glu Arg Pro Gln Ile Glu Ala Gly Ser Thr Glu Arg Ile Phe Leu Thr Val Ser

AAC TAC ATC TTC ACG GCC ATC TTC GTG GGC GAG ATG ACA TTG AAG GTA GTC TCG 4698  
 Asn Tyr Ile Phe Thr Ala Ile Phe Val Gly Glu Met Thr Leu Lys Val Val Ser

CTG GGC CTG TAC TTC GGC GAG CAG GCG TAC CTA CGC AGC AGC TGG AAC GTG CTG 4752  
 Leu Gly Leu Tyr Phe Gly Glu Gln Ala Tyr Leu Arg Ser Ser Trp Asn Val Leu

GAT GGC TTT CTT GTC TTC GTG TCC ATC ATC GAC ATC GTG GTG TCC CTG GCC TCA 4806  
 Asp Gly Phe Leu Val Phe Val Ser Ile Ile Asp Ile Val Val Ser Leu Ala Ser

GCC GGG GGA GCC AAG ATC TTG GGG GTC CTC CGA GTC TTG CGG CTC CTG CGC ACC 4860  
 Ala Gly Gly Ala Lys Ile Leu Gly Val Leu Arg Val Leu Arg Leu Leu Arg Thr

CTA CGC CCC CTG CGT GTC ATC AGC CGG GCG CCG GGC CTG AAG CTG GTG GTG GAG 4914  
 Leu Arg Pro Leu Arg Val Ile Ser Arg Ala Pro Gly Leu Lys Leu Val Val Glu

ACA CTC ATC TCC TCC CTC AAG CCC ATC GGC AAC ATC GTG CTC ATC TGC TGT GCC 4968  
 Thr Leu Ile Ser Ser Leu Lys Pro Ile Gly Asn Ile Val Leu Ile Cys Cys Ala

SUBSTITUTE SHEET (RULE 26)

- 33 -

TTC TTC ATC ATC TTT GGC ATC CTG GGA GTG CAG CTC TTC AAG GGC AAG TTC TAC 5022  
 Phe Phe Ile Ile Phe Gly Ile Leu Gly Val Gln Leu Phe Lys Gly Lys Phe Tyr

CAC TGT CTG GGC GTG GAC ACC CGC AAC ATC ACC AAC CGC TCG GAC TGC ATG GCC 5076  
 His Cys Leu Gly Val Asp Thr Arg Asn Ile Thr Asn Arg Ser Asp Cys Met Ala

GCC AAC TAC CGC TGG GTC CAT CAC AAA TAC AAC TTC GAC AAC CTG GGC CAG GCT 5130  
 Ala Asn Tyr Arg Trp Val His His Lys Tyr Asn Phe Asp Asn Leu Gly Gln Ala

CTG ATG TCC CTC TTT GTC CTG GCA TCC AAG GAT GGT TGG GTG AAC ATC ATG TAC 5185  
 Leu Met Ser Leu Phe Val Leu Ala Ser Lys Asp Gly Trp Val Asn Ile Met Tyr

AAT GGA CTG GAT GCT GTT GCT GTG GAC CAG CAG CCT GTG ACC AAC CAC AAC CCC 5238  
 Asn Gly Leu Asp Ala Val Ala Val Asp Gln Gln Pro Val Thr Asn His Asn Pro

TGG ATG CTG CTG TAC TTC ATC TCC TTC CTG CTC ATC GTC AGC TTC TTT GTG CTC 5292  
 Trp Met Leu Leu Tyr Phe Ile Ser Phe Leu Leu Ile Val Ser Phe Phe Val Leu

AAC ATG TTT GTG GGT GTC GTG GTG GAG AAC TTC CAC AAG TGC CGG CAG CAC CAG 5346  
 Asn Met Phe Val Gly Val Val Val Glu Asn Phe His Lys Cys Arg Gln His Gln

GAG GCT GAA GAG GCA CGG CGG CGT GAG GAG AAG CGG CTG CGG CGC CTG GAG AAG 5400  
 Glu Ala Glu Glu Ala Arg Arg Arg Glu Glu Lys Arg Leu Arg Arg Leu Glu Lys

AAG CGC CGG AAG GCC CAG CGG CTG CCC TAC TAT GCC ACC TAT TGT CAC ACC CGG 5454  
 Lys Arg Arg Lys Ala Gln Arg Leu Pro Tyr Tyr Ala Thr Tyr Cys His Thr Arg

CTG CTC ATC CAC TCC ATG TGC ACC AGC CAC TAC CTG GAC ATC TTC ATC ACC TTC 5508  
 Leu Leu Ile His Ser Met Cys Thr Ser His Tyr Leu Asp Ile Phe Ile Thr Phe

ATC ATC TGC CTC AAC GTG GTC ACC ATG TCC CTG GAG CAC TAC AAT CAG CCC ACG 5562  
 Ile Ile Cys Leu Asn Val Val Thr Met Ser Leu Glu His Tyr Asn Gln Pro Thr

## (2) INFORMATION FOR SEQ ID NO: 19:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 567

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

- 34 -

- (ii) MOLECULE TYPE: other nucleic acid  
 (iii) HYPOTHETICAL: no  
 (iv) ANTI-SENSE: no  
 (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: human  
 (ix) FEATURE: human alpha-I partial sequence  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATG CGG ATC CTG GTG AAC CTG CTC CTG GAC ACA CTG CCC ATG CTG GGG AAT GTC 54  
 Met Arg Ile Leu Val Asn Leu Leu Leu Asp Thr Leu Pro Met Leu Gly Asn Val

CTG CTG CTC TGC TTC TTT GTC TTC TTC ACC TTT GGC ATC ATA GGT GTG CAG CTC 108  
 Leu Leu Leu Cys Phe Phe Val Phe Phe Thr Phe Gly Ile Ile Gly Val Gln Leu

TGG GCG GGC CTG CTG CGT AAC CGC TGC TTC CTG GAG GAG AAC TTC ACC ATA CAA 162  
 Trp Ala Gly Leu Leu Arg Asn Arg Cys Phe Leu Glu Glu Asn Phe Thr Ile Gln

GGG GAT GTG GCC TTG CCC CCA TAC TAC CAG CCG GAG GAG GAT GAT GAG ATG CCC 216  
 Gly Asp Val Ala Leu Pro Pro Tyr Tyr Gln Pro Glu Glu Asp Asp Glu Met Pro

TTC ATC TGC TCC CTG TCG GGC GAC AAT GGG ATA ATG GGC TGC CAT GAG ATC CCC 270  
 Phe Ile Cys Ser Leu Ser Gly Asp Asn Gly Ile Met Gly Cys His Glu Ile Pro

CCG CTC AAG GAG CAG GGC CGT GAG TGC TGC CTG TCC AAG GAC GAC GTC TAC GAC 324  
 Pro Leu Lys Glu Gln Gly Arg Glu Cys Cys Leu Ser Lys Asp Asp Val Tyr Asp

TTT GGG GCG GGG CGC CAG GAC CTC AAT GCC AGC GGC CTC TGT GTC AAC TGG AAC 378  
 Phe Gly Ala Gly Arg Gln Asp Leu Asn Ala Ser Gly Leu Cys Val Asn Trp Asn

CGT TAC TAC AAT GTG TGC CGC ACG GGC AGC GCC AAC CCC CAC AAG GGT GCC ATC 432  
 Arg Tyr Tyr Asn Val Cys Arg Thr Gly Ser Ala Asn Pro His Lys Gly Ala Ile

AGC TTT GAC AAC ATC GGT TAT GCT TGG ATT GTC ATC TTC CAG GTG ATC ACT CTG 486  
 Ser Phe Asp Asn Ile Gly Tyr Ala Trp Ile Val Ile Phe Gln Val Ile Thr Leu

GAA GGC TGG GTG GCG ATC ATG TAC TAC GTG ATG GAT GCT CTC TCC TTC TAC AAC 540  
 Glu Gly Trp Val Ala Ile Met Tyr Tyr Val Met Asp Ala Leu Ser Phe Tyr Asn

TTC GTC TAC TTC ATC CTG CTT ATC ATA 567  
 Phe Val Tyr Phe Ile Leu Leu Ile Ile

- 35 -

## (2) INFORMATION FOR SEQ ID NO: 20:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 567

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: rat

(ix) FEATURE: rat alpha-I partial sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

ATG CGG ATC CTG GTG AAC CTG CTG CTC GAC ACG CTG CCC ATG CTG GGG AAC GTG 54  
Met Arg Ile Leu Val Asn Leu Leu Leu Asp Thr Leu Pro Met Leu Gly Asn Val

CTC CTG CTC TGT TTC TTC GTC TTC TTC ATC TTC GGC ATC ATT GGC GTG CAG CTC 108  
Leu Leu Leu Cys Phe Phe Val Phe Phe Ile Phe Gly Ile Ile Gly Val Gln Leu

TGG GCA GGC CTG CTA CGG AAC CGC TGC TTC CTG GAA GAA AAC TTC ACC ATA CAA 162  
Trp Ala Gly Leu Leu Arg Asn Arg Cys Phe Leu Glu Glu Asn Phe Thr Ile Gln

GGG GAT GTG GCC CTG CCC CCT TAT TAC CAA CCA GAG GAG GAT GAC GAG ATG CCC 216  
Gly Asp Val Ala Leu Pro Pro Tyr Tyr Gln Pro Glu Glu Asp Asp Glu Met Pro

TTT ATC TGC TCC CTG ACT GGG GAC AAT GGC ATC ATG GGC TGC CAC GAG ATC CCC 270  
Phe Ile Cys Ser Leu Thr Gly Asp Asn Gly Ile Met Gly Cys His Glu Ile Pro

CCA CTG AAG GAG CAG GGC CGG GAA TGC TGC CTG TCC AAA GAT GAT GTG TAT GAC 324  
Pro Leu Lys Glu Gln Gly Arg Glu Cys Cys Leu Ser Lys Asp Asp Val Tyr Asp

TTC GGG GCG GGG CGC CAG GAC CTC AAC GCC AGC GGT CTG TGC GTC AAC TGG AAC 378  
Phe Gly Ala Gly Arg Gln Asp Leu Asn Ala Ser Gly Leu Cys Val Asn Trp Asn

CGC TAC TAC AAC GTC TGC CGC ACG GGC AAC GCC AAC CCT CAC AAG GGC GCC ATC 432  
Arg Tyr Tyr Asn Val Cys Arg Thr Gly Asn Ala Asn Pro His Lys Gly Ala Ile

AAC TTT GAC AAC ATT GGC TAT GCC TGG ATT GTG ATT TTC CAG GTG ATC ACT CTG 486  
Asn Phe Asp Asn Ile Gly Tyr Ala Trp Ile Val Ile Phe Gln Val Ile Thr Leu

- 36 -

GAA GGC TGG GTG GAG ATC ATG TAC TAT GTG ATG GAC GCA CAT TCT TTC TAC AAC 540  
Glu Gly Trp Val Glu Ile Met Tyr Tyr Val Met Asp Ala His Ser Phe Tyr Asn .

TTC ATC TAC TTC ATC CTG CTT ATC ATA  
Phe Ile Tyr Phe Ile Leu Leu Ile Ile

567

- 37 -

### CLAIMS

1. An isolated DNA fragment comprising a sequence of nucleotides that encodes a calcium channel, wherein the sequence of nucleotides is selected from sequences of nucleotides encoding a protein including the sequence of amino acids set forth in SEQ ID. No. 19, and sequences of nucleotides that hybridize under non-stringent conditions to DNA encoding a protein including the sequence set forth in SEQ ID No. 19.
2. The DNA fragment of Claim 1, wherein the sequence of nucleotides is selected from sequences of nucleotides encoding a protein including the sequence of amino acids set forth in SEQ ID. No. 18, and sequences of nucleotides that hybridize under non-stringent conditions to DNA encoding a protein including the sequence set forth in SEQ ID No. 18.
3. The DNA fragment of Claim 1 or 2, wherein the calcium channel is a human neuronal calcium channel.
4. An isolated DNA fragment comprising a sequence of nucleotides that encodes a human calcium channel subunit, wherein the sequence of nucleotides is selected from sequences of nucleotides including the sequence set forth in SEQ ID No. 17.
5. A vertebrate expression vector containing the DNA fragment of any of Claims 1 to 4.
6. A eukaryotic cell transiently or stably transformed with the vertebrate expression vector of Claim 5, said cell expressing the calcium channel encoded by the DNA fragment.
7. A eukaryotic cell transiently or stably transformed with a heterologous DNA fragment according to any of Claims 1 to 4, said cell expressing the calcium channel encoded by the DNA fragment.

- 38 -

8. The eukaryotic cell of claim 6 or 7, wherein the cell is further transformed with and expresses an  $\alpha 2\delta$  or a  $\beta$  calcium channel subunit, or both.

9. A method for the production of the  $\alpha_{-11}$  protein of an animal cell calcium channel comprising, culturing the cell of Claim 6 or 7 under conditions whereby the DNA encoding the calcium channel subunit is expressed and the  $\alpha_{-11}$  subunit is produced.

10. A process for producing the eukaryotic cell that is transiently or stably transformed and expresses a calcium channel, comprising the step of introducing RNA or DNA having a sequence selected from among sequences that encode a protein including the sequence of amino acids set forth in SEQ ID. No. 19, and sequences of nucleotides that hybridize under non-stringent conditions to DNA encoding a protein including the sequence set forth in SEQ ID No. 19 and RNA or DNA encoding an  $\alpha 2\delta$  or  $\beta$  calcium channel subunit into a cell.

11. A method of identifying compounds capable of acting as agonists or antagonists for the  $\alpha_{-11}$  calcium channel, comprising contacting a cell according to claim 6 or 7 with an agent to be tested, and evaluating the interaction, if any, between the agent to be tested and the calcium channel.

12. An isolated DNA fragment having the sequence given by SEQ ID No. 19.

13. A method for mapping the distribution of calcium channel subunits within a tissue sample comprising the steps of exposing the tissue to a reagent comprising a directly or indirectly detectable label coupled to a DNA fragment comprising a sequence selected from among those sequences given by SEQ ID Nos. 13-20, and detecting reagent that has bound to the tissue.



1/3

## Figure 1 (Part I)

Query = C54D2.5 CE02562 CALCIUM CHANNEL ALPHA-1  
SUBUNIT LG:6

Database: Non-redundant Database of GenBank EST Division  
824,500 sequences; 302,742,428 total letters.

H55225 CHR220164 Homo sapiens genomic clone C22\_207 5'.  
Length = 168

Plus Strand HSPs:

Score = 136 (63.8 bits), Expect = 2.5e-10, P = 2.5e-10  
Identities = 23/31 (74%), Positives = 29/31 (93%), Frame = +1

Query: 440 VISLEGWTDIMYYVQDAHSFWNWIYFVLLIV 470  
VI LEGW IMYYV DAHSF N IYF LLI  
Sbjct: 1 VITLEGWVEIMYYVMDAHSFYNFYIFILLII 93

H55617 CHR220556 Homo sapiens genomic clone C22\_757 5'.  
Length = 98

Plus Strand HSPs:

Score = 102 (47.9 bits), Expect = 2.8e-05, P = 2.8e-05  
Identities = 19/23 (82%), Positives = 23/23 (100%), Frame = +2

Query: 243 NINLTAIRTVRVLRLRAVNRIP 265  
NINL AIRTVRVLRLPL A NR P  
Sbjct: 29 NINLSAIRTVRVLRLKAINRVP 97

2/3

## Figure 1 (Part II)

H55223 CHR220162 Homo sapiens genomic clone C22\_204 5'.

Length = 94

Plus Strand HSPs:

Score = 87 (40.8 bits), Expect = 0.0039, P = 0.0039

Identities = 14/19 (73%), Positives = 18/19 (94%), Frame = +2

Query: 154 MAVIMINCVTLGMYRPCED 172

M VI NCVTLGMY PC D

Sbjct: 2 MLVILLNCVTLGMYQPCDD 58

H55544 CHR220483 Homo sapiens genomic clone C22\_651 5'.

Length = 123

Plus Strand HSPs:

Score = 65 (30.5 bits), Expect = 3.8, P = 0.98

Identities = 12/23 (52%), Positives = 18/23 (78%), Frame = +1

Query: 246 LTAIRTVRVLRPLRAVNRIPSMR 268

RT R LRPLRA R MR

Sbjct: 55 IKSLRTLRLRLRPLRALSRLFEGMR 123

3/3

## Figure 1 (Part III)

F07776| HSC2HD061 H. sapiens partial cDNA sequence; clone c-2hd06

Length = 343

Plus Strand HSPs:

Score = 100 (46.9 bits), Expect = 0.00057, P = 0.00057  
Identities = 21/41 (51%), Positives = 31/41 (75%), Frame = +3

Query: 1480 PTIIRVMRVLRIARVLKLLKMAKGIRSLDDTVGEALPQVGN 1520  
PT+ RV+R+ RI R+L+L+K AKGIR+LL + +LP + N  
Sbjct: 57 PTLXRVIRLARIGRILRLIKGAKGIRTLLFALMMSLPALFN 179

# INTERNATIONAL SEARCH REPORT

Inte. onal Application No  
PCT/CA 98/00173

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C12N5/10 C1201/68 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TROFATTER JA ET AL: "An expression-independent catalog of genes from human chromosome 22." GENOME RES, OCT 1995, 5 (3) P214-24. UNITED STATES, XP002069420 see the whole document	1, 12
A	& EMBL database Accession number H55544 08-12-95 Trofatter j.a. et al. see the whole document	1, 12
A	WO 95 04144 A (NEUREX CORP) 9 February 1995 cited in the application see claims 1-10	1, 5, 6, 9-12

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

25 June 1998

Date of mailing of the international search report

09/07/1998

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## INTERNATIONAL SEARCH REPORT

### Information on patent family members

International Application No.

PCT/CA 98/00173

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9504144 A	09-02-1995	EP 0778890 A JP 9501051 T	18-06-1997 04-02-1997
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